Unhairing of Hides and Skins by Amylase Preparations

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The use of enzymes for unhairing hides and skins dates back to antiquity, for the bacteria that are active in the old-time sweating process accomplish their action by means of the enzymes which they elaborate. Isolated enzymes were first used successfully by Otto Röhm in 1910 [4] when he found that the use of pancreatic enzymes for bating could be extended to unhairing if the skins were first swollen in an alakli solution such as soda, ammonia, or borax and neutralized with sodium bicarbonate.

Since this original work of Röhm's numerous attempts have been made to utilize plant, animal, and microbial enzymes for unhairing hides and skins. Green [3] has recently reviewed the literature including the patent literature, and, according to him, a mold enzyme process is now in use in Germany for the manufacture of glacé kid. This is the only instance of the commercial use of isolated enzymes that has been noted. Most tanners believe that enzymes do not give uniform hair loosening or are difficult to control or are too costly.

In 1953 Burton, Reed and Flint [1] suggested a novel approach to the unhairing problem. They stated that all fibrous protein structures are in close association with mucoids; thus such material is present in the spaces between the corium fibers and especially at the epidermal-corium junction and around the hair follicles. They suggested that mucoid material may play an important part in the lime yard processes and that soaking back, unhairing and bating may, in essence, be the removal of this material. On the basis of this thesis they tried what they termed mucolytic enzymes such as pectinase, diastase and pancreatic elastase and found that these enzymes removed the hair

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from fresh calfskin at room temperature in about 3 days. Testicular hyaluronidase was incapable of unhairing fresh calfskin over the pH range of 5.2 to 7.5. No details are given as to how the experiments were conducted.

Not long after the paper by Burton, Reed and Flint (loc. cit.) appeared, Gillespie [2] published a paper in which he described the depilation of sheepskins by enzymes from certain fungi. Commercial preparations of papain, trypsin, pancreatin, chymotrypsin, pepsin, erepsin, amylase, lipase, malt diastase, takadiastase, arazyme N and oropon F were also tried. None of these commercial preparations showed any activity in the presence or absence of activators. The enzymes were applied to the flesh side of the sheepskins in the form of a clay paste. It is doubtful if Burton et al. (loc. cit.) used this procedure, so their results may not be comparable. Gillespie suggested that the unhairing action found by Burton et al. (loc. cit.) was due to the action of contaminating bacteria. He stated that pectinases and diastases would not be expected to attack the mucoid materials as postulated by Burton et al. (loc. cit.) nor would mucolytic enzymes be expected to be present in such preparations.

Experimental

Preliminary trials were made with commercial amylase preparations on salted hide. The preparations tried are listed in Table I. This salted hide was used because it was immediately available although it had been in storage for some time at 35 - 40° F. and was showing hair slip in spots. Only pieces that had tight hair were used for testing. Although there were differences in the effectiveness of various samples of enzymes, all caused some hair loosening and some were very effective, causing the hair to become extemely loose in 16 hours. It was evident very early that bacterial growth must be prevented because bacteria could cause hair loosening and thus invalidate the results.

TABLE I

Amylase Preparations Tried as Unhairing Agents

Enzyme Preparation	Source	Origin, where known
Special Diastase 160	Takamine Laboratories	——
RT-44	" "	Bacterial
Clarase 300	" "	Fungal
Pancreatin 3 USP	" "	Animal
Rhozyme 51	Rohm and Haas Company	——
Rhozyme DX	" " " "	——
Rhozyme S	" " " "	——
Mylase SA	Wallerstein Laboratories	Fungal (Aspergillus oryzae)
Enzyme W3F	" "	——
Enzyme 164	" "	——
Mylase L-1	" "	——

Several antiseptics were tried including the usual toluene. The most effective was phenyl mercuric acetate in a concentration of 0.15 gram per liter. Although sodium chloride precipitates this material, it still maintains sterility for at least 3 or 4 days. This antiseptic was used in all tests.

Since Burton, Reed and Flint (loc. cit.) had used fresh hide, it was deemed advisable to try the process on fresh, unsalted hide. A medium weight cowhide was obtained from a slaughterhouse shortly after it was flayed. It was washed in a drum for 1 hour, fleshed, cut in strips and quick frozen in a deep freeze chest. Strips were removed and thawed as needed.

When this fresh hide (before or after freezing) was treated with the commercial amylase preparations there was little or no hair loosening. It was soon discovered that pretreatment of fresh hide with sodium chloride solution would render it susceptible to action of some diastase preparations and that 2 to 3 per cent salt was more effective than 5, 10 or 20 per cent.

The following experiment illustrates the effect of pretreatment with salt. Four 1½″ x 3″ pieces of fresh, frozen cowhide were thawed and weighed. Each piece weighed 30 grams. One piece was placed in 200 ml. of each of the solutions shown in Table II. All the solutions contained 0.15 gram per liter of phenyl mercuric acetate. The solutions and hide pieces were in rectangular pint jars, which were placed in a constant temperature oven equipped with a device for rotating the jars at 9 - 10 RPM on their long axis. The temperature was kept at 38° C. After 24 hours' incubation the solutions were drained from the jars of treatment numbers 1 and 2 (Table II) and a 0.25 per cent

TABLE II

Effect of Sodium Chloride on the Unhairing Action of "Special Diastase"

		Degree of Hair Loosening* at Hours Incubation		
No.	Treatment	24	48	72
1	Antiseptic 24 hours followed by 0.25% diastase	0	+	++
2	2% NaCl 24 hrs. followed by 0.25% diastase	0	+++	+++++
3	0.25% diastase	0	0	+
4	2% NaCl + 0.25% diastase	0	++	+++

*Rated 0 to +++++, where +++++ = hair completely loose.

diastase preparation* was added. There was little hair loosening except where salt was used. Pretreatment with salt was more effective than adding it with the enzyme. This may be due to the fact that a certain time is required for the salt to act before the enzyme can act. During this time the enzyme may be losing activity. In some experiments a slight hair loosening was produced simply by washing the hide in the antiseptic solution for several days.

*The preparation used was "Special Diastase 160" sold by the Takamine Laboratories.** It is described as a "Unique formulation of various diastases" . . . containing "buffering, activating and stabilizing salts".

**In mentioning trade names the Eastern Utilization Research Branch, United State Department of Agriculture does not in any way guarantee the products nor are they recommended in preference to others not mentioned.

The possibility of a depilatory enzyme being present in the skin is thus suggested. This point is under investigation.

Numerous attempts have been made to produce this enzyme unhairing without sodium chloride. Activators such as cysteine, sodium sulfite, and sodium sulfide, which were used by Gillespie (loc. cit.) were tried but had no effect. Pretreatment with dilute (1-2%) urea was almost as effective as sodium chloride.

On the theory that the enzyme must penetrate to the site of action from the flesh side (as it must in Gillespie's test), successive layers of corium were split off. There was no apparent effect upon the hair loosening by the enzyme whether most of the corium had been removed or not.

The effect of agitation and flexing is shown in Table III. Fresh, frozen calfskin was thawed and placed in a 2 per cent sodium chloride solution containing 0.15 gram per liter of phenyl mercuric acetate and allowed to stand for 2 days. Another piece of fresh, frozen calfskin was taken from the freezer to serve as the unsalted control. One piece of salt-treated and one of fresh skin were incubated under the conditions shown in Table III in solutions of a 0.25 per cent diastase preparation.* The effects of salt treatment and flexing are shown very well by these results.

TABLE III

The Effect of Agitation and Flexing on the Unhairing Action of "Special Diastase"* on Calfskin

		Degree of Hair Loosening at Hours Incubation		
No.	Treatment	24 Temp. 24° C.	48 Temp. 24° C.	72 Temp.**
1	Stationary			
	Salt treated	0	0	+
	Not salt treated	0	0	0
2	Agitated in "Rotor oven"			
	Salt treated	0	0	+++++
	Not salt treated	0	0	++
3	Flexed at 88 strokes per min.			
	Salt treated	+	+	+++++
	Not salt treated	0	0	0

*0.25% solution.

**Since the action was so slow at 24° C., the temperature was increased to 39° in the agitated samples and to 27° in the others.

Summary

At present it appears that enzymes are unable to penetrate to the hair roots without the previous action of sodium chloride, urea or perhaps other materials. The function of these substances, is, as yet, not clear, but it appears that they dissolve some constituent of the hide which prevents the action

of the enzyme. Also, it is not known as yet what enzymes are responsible Commercial amylases are certainly not single enzymes, and it may well be that on entirely unsuspected enzyme is responsible for the depilatory action. It is hoped that light can be thrown on this question by future work. It has been found that some reducing substance, presumably sugar, is produced by the action of enzymes on hides. So far we have not been able to demonstrate any correlation between this "sugar" release and the looseness of the hair.

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